REMARKS

Reconsideration of this patent application is respectfully requested in view of the foregoing amendments, and the following remarks.

The present application teaches a process for destabilizing a viral quasi-species distribution without inducing resistance with the point of attack being the replication system of the viruses.

(d) Therefore, the present application teaches the application of agents with inherent properties.
There is not any substances which can be used in the scope of the invention. The present application refers only to substances which affect the replication negatively by means of a higher rate of misincorporation but without effecting/decreasing the efficiency of the replication. Chemical substances inhibiting the replication machinery per se must be avoided.

On page 8, second paragraph (=[0025]): "An important criterion of the process according to the invention is the demand that the replication of the quasi-species-

distribution, which has the replication system with higher error misincorporation than the natural distribution, proceeds at least as efficiently as the replication of the quasi-species-distribution of the viral wild-type. "

Page 20, last paragraph (= [0065] in publication: "On the other hand, the strategy according to the invention, which in the meaning of an information conservation is producing mutants which are too strongly subject to errors, leads via a period of longer lasting infection to a degeneration of the quasi-species-distribution, corresponding to the wild-type virus and, therefore, to the extinction thereof. The error rate of the virus replication will be elevated according to the invention above the evolutionary admissible threshold, whereby the replication must not be inhibited but at least should proceed as efficient as the one of the wild-type virus."

The amendments to this patent application are as follows. Claim 69 has been withdrawn from further examination, and this has been so indicated for claim 69, based upon the previous Restriction Requirement. The present Specification has been amended on Pages 1, 5 and 14

to recite the required Section Headings. A Brief Description for FIGS. 5 and 6 has now been provided on Page 14 of the present Specification.

Claims 61 and 72 were objected to because allegedly each claim recited the phrase "a higher error rate of rate of misincorporation." Claim 61 has been revised to correct this error in terminology. On the other hand, claim 72 does not recite this terminology.

In claim 61 the objected to phrase has been amended by deletion of the repeated words "rate of." With regard to the objection of claims 61 and 72 as being not enabled, it is believed that the entire invention is sufficiently disclosed so that a skilled person in the art is enabled to practice the invention. The enclosed Table provides information to rebut the objection of lack of enablement by the Patent Examiner (with reference to the numbering of the published document US 2002/0107220).

The claims 61 and 72 are directed to a process or method which is a particular embodiment of the invention and its principles. Having understood the principles and concept of the present invention there is no undue burden for the any person skilled in the art to find the respective concrete features following the lines as given in the

specification. In the conception of the invention the skilled person knows perfectly how to arrange for the several process steps of claims 61 and 72.

The Examiner cites Ex parte Forman and alleges (a) that in order to practice the invention the practitioner must be able to apply any substances to the effected target cells. This is too simplifying since according to the understanding of a person skilled in the art that only substances are involved having the ability to interfere with the replication system of the Infectious system by means of a higher rate of misincorporation but without decreasing the efficiency of replication. [0065: "The error rate of the virus replication will be elevated according to the invention above the evolutionary admissible - threshold, whereby the replication must not be inhibited but at least should proceed as efficient as one of the wild type virus."] (see (f) # 4 and 5).

Concerning (b) it is noted that the Patent Examiner acknowledges guidance with regard to AZT. Thus, a process is described which can be performed and followed in an analogous process for other substances having the ability to interfere with the replication system of an infectious system. (c) There was an objection to the allegedly missing working examples for the treatment of effected target cells

with any substances. First of all not any substance can be used but only a substance which interferes with the replication system of an infectious system in the scope of the invention as stated above, with guidance given in the Specification (see information in Table). Furthermore, according to case law enablement does not depend on a specific wording example.

Also in (d) the objection is based on the assumption that the target cell can be treated with any substance. However, the skilled person knows perfectly well that not any substance can be used, but only those which will interfere with the infectious system by an increased rate of misincorporation but still maintained efficiency of the replication system.

Comments for (e) The document US 4,536,398 teaches the substance SF-2140 as a new antiviral agent. SF-2140 was also found to have antibacterial activity. The examples demonstrate a proliferation inhibiting activity of this new substance versus influenza viruses. However, the document does not teach the application of this antiviral substance in order to overcome the problem of resistance phenomena. This new substance may be an agent potentially applicable in the present application, if it will be further characterized in the scope of the present invention, i.e. effecting

defects in the replication without decrease of the efficiency of the replication.

The specification clearly indicates how to use the invention. Starting page 21, second paragraph (=[0066]), there is an exemplified protocol depicting the step-by step handling how to find out suitable agents. Incubating the virus replicating system (i.e. the cultured infected cells) with potential agents of different concentrations, the influence of chemical substances on the error rate of the replication machinery can be monitored and an effective concentration can be determined. Using, for example, temperature gradient gel electrophoresis (cited patent application PCT/EP90/01366), altered nucleic acids can be identified and the degree of alteration can be quantified.

Page 20, last paragraph (= [0065] in publication: "On the other hand, the strategy according to the invention, which in the meaning of an information conservation is producing mutants which are too strongly subject to errors, leads via a period of longer lasting infection to a degeneration of the quasi-species-distribution, corresponding to the wild-type virus and, therefore, to the extinction thereof. The error rate of the virus replication will be elevated according to the invention above the

evolutionary admissible threshold, whereby the replication must not be inhibited but at least should proceed as efficient as the one of the wild-type virus."

(f) Concerns the level of skill of those in the art. The Patent Examiner agrees that the level of skill in molecular biology is high. Thus, also the people working in molecular biology have high skills so that the level of the person skilled in the art is comparatively high as far as the knowledge about molecular biology technology is concerned. It is noted that the Patent Examiner is silent with respect to item (g) of the Forman Decision. There is no speculation of the Examiner regarding the predictability of the art. With regard to (h) it may be stated that broad claims do not necessarily mean that the invention cannot be practised due to lack of enablement.

In 7.a, the Patent Examiner objects to the missing process steps. This has been corrected by amendments to the claims, wherein process steps have been positively recited wherever appropriate.

In 7.b, the wording of claim 47 has been changed by deleting "wild type" after "misincorporation of the viral" and inserting "of a wild-type" after the term "replication system" in the line "misincorporation of the viral . . . .

replication system."

In 7.c, an amendment has been made to claim 52 to provide the required antecedent basis.

In 7.d, the wording "RNA or DNA" has been delected from claim 53. It was intended to point out that the replication systems are based on RNA or DNA, however, this is self-explanatory from the remaining claim language of claim 53.

In 7.e, the word "the" has been deleted before "gene therapy" in claim 54.

In 7.f, the term "superinfection" in claim 55, means that a cell which was infected with an infectious system is further infected with the same organism having an infectious system which has a defective replication system. This leads to the infiltration of the defective replication system into the target cell.

In 7.g, with regard to the characteristic superiority parameter "s" in claim 60, it is in the Specification where the superiority factor is defined.. With regard to the allegedly lacking of antecedent basis, the deletion of the term "the" and changing it to "a" overcomes this objection.

With regard to the term "combination of the replication system" it should be pointed out that the phrase is not a single replication system. Instead a combination is a replication system and one or more nucleases or ribozymes and/or antisense-RNA.

In 7.h, claims 61 and 72 have been amended to provide the proper antecedent basis.

In 7.i, claim 64 has been amended to provide the proper antecedent basis.

No. 7 on page 7 obviously means 8. The references which were provided were intended to show the knowledge of the skilled person at the day of filing of the patent application for the present invention. Significant information which allegedly is not disclosed in an enabling way in the Specification can be derived from the respective references.

For all these reasons, the present invention, the Specification, and all the claims are firmly believed to be in complete compliance with all of the requirements of 35 U.S.C. 112. Withdrawal of this ground of rejection is respectfully requested.

Respectfully submitted,

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Enclosures: 1)Table: Disclosure in Specification
2)Copy of Petition 3 Month Extension of Time

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as first class mail in an envelope addressed to: Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on June 4, 2003.

Maria Guastella

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Table: **Disclosure in the Specification** (Citations refer to passage numbering of the published document US 2002/0107220 A1 relating to this application)

| # | step | | Ref. | Text passage – wording |
|---|---|---|---|---|
| 1 | **Background knowledge** | Concept of quasi-species and error threshold | [0004] lower part | ...the viral genome is **macroscopically defined and determinable as consensus sequence, but it is microscopically present in the form of a mixture of many mutants which are permanently in competition with one another.** This competition maintains the genetic information of the virus in a dynamic equilibrium of mutation and selection. **The genetic information modified by mutation is always re-established by selection, in such a way that the infection chain is maintained** |
| | | | [0005] | By using a **quasi-species-distribution**, a population of microorganisms **can adapt extremely rapidly** to different environmental conditions |
| | | | [0006] | The viral population can not stand to an increase of the rate of misincorporation of the viral replication system, e.g., **in the case when the rate of misincorporation surpasses the theoretical error threshold belonging to the replication system. The portion of defect descendants will become that high that the infection chain can no more be maintained** for a long time by viruses which still are infective. |
| | | | [0008] | Theoretical calculations support the findings that a distribution of quasi-species-information can be maintained only in a stable way during facultative periods of time **as long as a certain error threshold of the replication apparatus is not exceeded.**[...] The calculation predicts **a diffluence of information and therefore, the termination of a quasi-species-distribution in the vicinity of the wild-type sequence, if this error threshold is exceeded.** |
| | | | [0016] | To begin with, Eigen et al. (1971) deduce from pure theoretical considerations **the error threshold of a replicative system.** A relation between the quantities q, m, s is established: m=ln s(1-q).sup.-1 |
| | | | [0023] | When m=ln s(1-q).sup.-1, then the actual rate of misincorporation of the natural system is equal to the theoretical error threshold of this system. **Now, when exceeding this theoretical error threshold, then mutants of the quasi-species-distribution are formed in a progressive way, a fact that is finally leading to an enlargement of the quasi-species-distribution** |
| | | | [0024] lower part | **... The mutant spectrum is enlarged when the replication is subject to greater errors.** When the replication is beyond the allowed error threshold, the quasi-species-distribution attains no new equilibrium. The distribution will become continuously broader with progressive replication (t1, t2, t3), **until finally the information flows off completely.** It comes to the "error catastrophy". **The virus is extincted.** |
| | | | [0013] | The theoretically deduced relation of the error threshold (see below) constitutes the parameter s **superiority parameter (superiority) and (1-q) (rate of misincorportion). These parameters constitute the basis of the process according to the invention** for exceeding the maximum permissible rate of error, the theoretical error threshold. In this way, the quasi-species-distribution is becoming unstable. |
| | | | | |
| | | Reflections to the field of | [0006] | The viral population can not stand to an increase of the rate of misincorporation of the viral replication |

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| | | viruses | | system, e.g., in the case when the rate of misincorporation surpasses the theoretical error threshold belonging to the replication system. The portion of defect descendants will become that high that the infection chain can no more be maintained for a long time by viruses which still are infective. |
| | | | [0027] | The process according to the invention is based on the surprising finding that in the meaning of an evolutionary virus distribution, a defective degeneration at a high amplification rate is as unfavorable as the inhibition of the virus replication. |
| | | | | |
| | | to be avoided with the present application | [0009] | ...the dilemma in which the classical search for active agents is involved by screening of the active materials. [...] Therefore, the common conceptions of today for the so called "drug-screening" are even dangerous because of the permanent danger that new, possibly even stronger pathogenic viruses, are induced by this undesired selection pressure. |
| | | | [0021] | Previous antiviral strategies try to suppress the replication per se. The hereby presented process is based on knowledge derived from the relation of the error threshold. |
| | | | | |
| 2 | General Object/ Intention | | [0010] | The object of the present invention is to provide a generally applicable process by which the virus population lose certain pathogenic properties, particularly the property of infectiousness. Related to this is the *provision of an agent by which viral infections can be treated therapeutically and prophylactically without stimulating the formation of resistent virus populations.* |
| 3 | Approach: | Defective replication system with | [0014] | The process according to the invention exploits the instabilisation of a viral quasi-species-distribution which is based upon the replication of this distribution with an incorrect replication system. Thereby, the incorrect replication system has a rate of misincorporation exceeding the rate of misincorporation of the wild-type replication system. The rate of misincorporation is a measure of accuracy by which a given viral replication system of a quasi-species-distribution can replicate a genome. |
| 4 | Replication system | Definition | [0032] | RNA or DNA polymerases or co-factors of RNA or DNA polymerases are used as preferred replication systems. |
| | | Requirements to defective replication system | [0021] | A central parameter is, as will be seen later on, the parameter q which can be diminished to lower values by measures according to the invention without influencing the efficiency of the replication as a whole. |
| | | | [0025] | An important criterion of the process according to the invention is the demand that the replication of the quasi-species-distribution, which has the replication system with higher error misincorporation than the natural distribution, proceeds at least as efficiently as the replication of the quasi-species-distribution of the viral wild-type |
| | | | [0014] | The process according to the invention exploits the instabilisation of a viral quasi-species- |

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| | | | | **distribution which is based upon the replication of this distribution with an incorrect replication system.** Thereby, the **incorrect replication system has a rate of misincorporation exceeding the rate of misincorporation of the wild-type replication system.** The rate of misincorporation is a measure of accuracy by which a given viral replication system of a quasi-species-distribution can replicate a genome. |
| | | | [0026] | A second central prerequisite of the process according to the invention is that the **polymerase or polymerase subunit replicates specifically the correspondent viruses for the respective virus beyond the error threshold, without being less efficient in this case than the correspondent wild-type component.** [...]Therefore, it is necessary that the rate constants for the processes of initiation, elongation and the termination do not differ considerably from the respective constants of the wild-type units |
| | | | [0065] | On the other hand, the **strategy according to the invention**, which in the meaning of **an information conservation is producing mutants which are too strongly subject to errors**, leads via a period of longer lasting infection to a degeneration of the quasi-species-distribution, corresponding to the wild-type virus and, therefore, to the extinction thereof.<br>-The error rate of the **virus replication will be elevated** according to the invention **above the evolutionary admissible threshold**,<br>- whereby the **replication must not be inhibited** but at least should proceed as efficient as the one of the wild-type virus. |
| | | | | |
| | | Induction of defective replication system | [0029] | The **defective replication** of the viral nucleic acid can be **induced**, according to the invention, preferably **by action of a chemical substance.** |
| | | | | |
| 5 | **Agent** | Features of a suitable agent | 0029] | In this case, the **substance can act as an antimetabolite or allosteric effector of the replication system** |
| | | | [0029] | The substance is preferably of **such a kind that it does not interact with the cellular enzyme system**, in order to prevent, optionally, the toxic side-effects. |
| | | | [0010] | Related to this is the provision of an **agent by which viral infections can be treated therapeutically and prophylactically without stimulating the formation of resistent virus populations.** |
| | | | [0065], middle | This can be accomplished by various measures. In this way, **an active agent which interferes with a replication process in a way that it comes to a specific elevation of the rate of misincorporation of the viral replication system, can be used.** |
| | | | [0002] | A suitable point of attack of the reproduction of the virus is the specific virus replication system or one of the components thereof. In such a way, the **viral replication apparatus can be inhibited by** |

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| | | | | **antimetabolites as AZT (HIV, AIDS) or Acyclovir (HSV, herpes).** |
| | | Screening for suitable agents | [0065], middle | .. By using the **prior antiviral screening systems such an active agent** cannot **be identified.** → The use of a **system such as it is described according to the invention, in combination with in vitro-cell cultures,** however, permits an effective screening to such mutants of a polymerase from samples of defective genome populations, as described above for E. coli-viruses. |
| **6** | **Exemplified Protocol for the provision of suitable agents** | | [0066] upper part | **Example: The screening to HIV:**<br>- target cells like peripheral blood cells or transformed lymphocyte cells like HUT 78 infected with cloned HIV-variants.<br>- Incubation of the virus replicating system is with potential active agents of different concentrations.<br>- After cultivation, in vitro amplification of representative gene locus of the virus-RNA or virus-DNA with a polymerase having a low error rate.<br>- Hybridisation of the amplification product with a responsive, cloned probe of the starting variants<br>- Examined by a detection system as described **in PCT/EP 90/01366 (→ temperature gradient gel electrophoresis)**<br>- Result of the analysis:<br>- → Presence of incorrect virus variants which are amplified within the cells.<br>- → In this way, such active agents can be detected that exceed the error threshold, which, however, do not prevent a replication and not necessarily influence measurably the infectivity.<br>- → Conclusion: Expectation from such active agents to act allosterically. Therefore, they should also not interact inevitably with cellular polymerase systems, contrary to conceptions which are based on the principle of antimetabolites, e.g. nucleotide analogue as AZT. |
| | | | [0073] | → In an analogous manner, a **population of specific sequences** can be tested in the meaning according to the invention **which are formed by help of the procured, defective polymerase.** These fragments can be tested either directly in the temperature gradient electrophorese gel system, or after amplification as by PCR-reaction with or without previous transcription in DNA by a reverse-transcriptase. |

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| 7 | **Screening** | for suitable polymeraes (defective replication system): | [0069] | Several processes of different complexity are available in order to find out a suitable polymerase from a natural or artificial spectrum of mutants of a polymerase: |
| | | **a) first selection criteria**<br>- morphological diversity of the plaques<br>- The magnitude of the plaques | [0070] | It has to be tested, if a different morphology of the above described Q.beta.-plaques can be attributed to a defective replicating polymerase (replicase). A simple genetic test is a plaque purification of the morphologically changed plaques. When the morphological peculiarity is maintained more or less uniformly, this fact will not indicate a mutation in the polymerase-gene section but in another segment of the polycistronic genome. **However, when again plaques of a greater morphological diversity are formed, it will be a strong indication to a defectively working polymerase. The magnitude of the plaques in turn, can be rated as an indication of rapid synthesis kinetics.** It was to expect that greater plaques also have a more rapid replicase. Thereby, the expenditure in relation to the molecular-biologically characterized finding of the desired species can be reduced |
| | | **b) Second analysis**<br>- temperature gradient gel electrophoresis (TGGE) | [0071] | It is possible, to reliably analyze an uncertain plaque by the technique of the temperature gradient gel electrophoresis, if it actually contains phages having a replicase with greater rate of misincorporation. |
| | | | | |
| 8 | **TGGE** | Features | [0072] | The temperature gradient gel electrophoresis can **detect mutations in a certain nucleic acid segment** in a highly sensitive manner. In this way, also mutants can be found in a population of sequences. |
| | | | [0076] | As described by Henco et al. [Nucleic Acids Res. (1990)], the temperature gradient gel electrophorese system can be used for the exact quantification of nucleic acids in a probe, whereby the accuracy being .+-.15%. |
| | | | | |
| | | Message/Result of TGGE | [0073] | **From the intensity of the mutant bands in relation to the intensity of the homoduplex** band can be **concluded to the relative frequency of the mutants, respectively, to the rate of misincorporation of the replicating enzyme system** (W. Thilly). |
| | | | [0074] | A radio-labelled segment can preferably be added to the mixture, the frequency of which is identical to the one of the homoduplex in order to estimate the error rate of a replicase. In this way, the radioactivities of the homoduplex band and that of the heteroduplexes can be determined by counting after the denaturation and renaturation being realized (FIG. 5). The ratio of the measured activities is then a direct measure for the error rate of the replicating system, respectively, of the replicase. |
| | | | [0075] | [...] **an enlargement of the quasi-species-distribution occurs when the defective replication process according to the invention** is participating. This will be seen in the temperature gradient gel electrophorese analysis by **an increasing rate of the band intensity of the mutant spectrum in relation to the band having the greatest relative frequency, previously called wild-type.** |

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| | | | [0076] | The temperature gradient gel electrophorese system can also be used to measure the efficiency, i.e. the replication velocity in the above described meaning, respectively, to compare it with the one of the wt-enzyme. |
| | | Kind of samples subjected to TGGE | [0073] | These fragments can be **tested either directly in the temperature gradient electrophorese gel system, or after amplification as by PCR-reaction with or without previous transcription in DNA by a reverse-transcriptase** |
| | | | [0074] | **A radio-labelled segment can preferably be added to the mixture** |
| 8 | **Prophylaxis** | | [0066] lower part | ... process according to the invention can be a used in a particularly preferred embodiment when transgenic systems are used, as it is already practiced in the plant sector with the coat-protein production. **Stem cells without differentiated potential receiver cells of infected or endangered patients can be transformed with a gene for a virus polymerase (RNA-replicase, reverse transcriptase or DNA-dependent DNA-polymerase) with elevated error rate, which can be obtained by the above mentioned screening process**. Whatever virus mutants produces a defective replication, they will encounter a target cell which already provides the viral replication system with elevated rate of misincorporation or decisive co-factors or subunits thereof. All of the virus variants are replicated above their characteristic error threshold. In this way, no virus variant is able to develop resistances. The information will flow off with further amplification. The process will be the more efficient the less the virus amplification is inhibited per se |
| 9 | **Link to other application/ Target cells** | | [0077] | The described processes can be used analogously for the discovery and classification of polymerase mutants of other virus/host systems which are applicable according to the invention. When in bacteria, as in the case of **tobacco mosaic virus (TMV)--in analogy to the plaque formation**--clonal, virus **caused lesions can be detected in leafs**, exits the possibility to conclude from the morphology of single lesions to the presence of viral polymerase mutants with elevated rate of misincorporation. |